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TRANSLATION OF PCT/EP00/03860**REPLACED BY
ART 34 AMDT**

**A DEVICE FOR PERFORMING
ANALYSIS ON CELL CULTURES**

DESCRIPTION

The invention relates to a device for the carrying out investigations of cell cultures, which are in a liquid culture medium, wherein the device possesses at least one receptacle for the culture medium with the cell culture, and wherein one or more measuring apparatuses and/or sensors for measuring the cell culture are provided.

In the case of such a conventional device, fresh culture medium is admitted to the cells in a specified timed sequence, or an active agent dissolved in this culture medium is added. Also, exhausted medium is correspondingly removed from the cell culture zone. A substantial regeneration of the culture medium, for example by means of an appropriate fluid system, produces a far-reaching, constant, physiological milieu. Easily dissociating agents, which are added to the nutrient material, likewise can be regenerated. The added medium and the cell culture zone itself must be protected from contamination by microorganisms and from excessive evaporation. These are important preliminary measures for the sensitive measurement of cellular reactions.

Common to all cell culture operations, is a surface on the bottom for the cell storage and cell growth as well as wall surfaces which form a trough for the containment of the culture medium. The culture medium must be regenerated at regular periods, since the waste products of the cell metabolism accumulate. Nutrient substances are consumed and biological active materials decline in their activity in the course of time.

EP 0 394 406 has previously disclosed an apparatus for the fluid handling of cell cultures in combination with sensors. This apparatus possesses a tightly closed, small

volume perfusion chamber, in which the cells are cultivated and which chamber is simultaneously fitted out with a sensor. The chamber possesses both an inlet and outlet channel. The driving power is a liquid pump, which circulates the culture medium through the perfusion chamber. At periodic, sequential intervals, there follow phases with perfusion and phases without perfusion. The phases with perfusion serve for the regeneration of the culture medium, the phases without perfusion promote the measurement procedure, that is, the direct monitoring of the extra cellular acidification in the perfusion chamber.

The above procedure is disadvantageous, however, in that a considerable investment in apparative equipment is necessary, since a liquid pump, valves for controlling the fluid flow, as well as sufficient hose for connections are required. A further disadvantage is, that a certain propensity for the formation of air bubbles in the perfusion chamber is present, and the exclusion of this disadvantage is again associated with considerable expense in time and money. For this situation devices are necessary for partial degassing of the medium, which must be coupled with the cell cultures. This again increases the expense. Finally, a relatively great expenditure of operational effort is necessary, in order to obtain an air bubble free and air tight, water tight assembly of the system. An especially disadvantageous situation comes to light when a multiplicity of parallel samples are to be tested, and such sampling is the rule in practice.

Thus, the object of the present invention is first, the creation of a comparatively simple arrangement for the regeneration of cell culture solutions and second to establish the use of smaller measurement volumes. At the same time, allowance should be made, to accommodate the required geometrical ambient conditions of cell culture.

The proposal for the achievement of these objectives is that a separating element is provided, which closely approaches the cell culture found on a bottom of a receptacle, thereby displacing a portion of the volume of the liquid medium which covers the cell

culture and in this position, from above the culture medium, confines a reaction space.

In an advantageous manner, in this arrangement, one or more sensors and/or measurement apparatuses are placed in the zone of this reaction space. Further, in accordance with a preferred embodiment of the separating element, the separating element can be moved back and forth between a position proximal to the bottom and a position distal therefrom. In the position approaching the bottom, the separating element will then border the reaction space.

In the position near to the bottom, the separating element forms a boundary for a small volume reaction space, which serves for diffusion-restriction for substances, which are formed, which are products of the cells or have been consumed.

With this measure, in accordance with the invention, a small volume reaction space can be realized, in which the measurable material changes which are a condition of the cell metabolism can proceed much more swiftly than in a large volume. From the speed of the change of the pH value or the oxygen partial pressure in one unit of time interval, information can be gathered in regard to the activity of the cellular metabolism.

One embodiment of the invention provides, that culture zones, are separated from one another, especially separated by structured, cell rejecting installed coatings or by means of a three dimensional structuring of the bottom with recesses or elevations formed in the culture zones which lie between. The culture medium advantageously is present in drop form.

With the separating element, which is preferably shaped like a hand stamp, and which exhibits an underside, flat surface, the drop can be compressed from above and a partial volume is displaced to the sides. This continues up to the point, where underneath the separating element, only a thin, liquid film remains. This thin film then forms the reaction space with a micro reaction volume of the culture medium.

Claims

What is claimed is:

1. A device for the carrying out of investigations on cell cultures, which take place in a liquid culture medium, wherein the device possesses at least one receptacle for the culture medium and the cell culture and wherein one or more measurement apparatuses and/or sensors are provided for measurements on the cell culture, therein characterized, in that a separating element (7a) is provided, which can so closely approach the cell culture (2) which is found in a receptacle having a bottom for the purpose of displacing a partial volume of the liquid culture medium (4) which is covering the cell culture and in this position above the culture medium (4) the separating element 7 confiningly borders a reaction space 8a.
2. A device in accordance with Claim 1, characterized in that the separating element (7a), is movable back and forth between a position proximal to the bottom and a position distal from the bottom and in the position proximal to the bottom confiningly borders the reaction space 8a.
3. A device in accordance with Claim 1 or 2, characterized in that at least one separating element for one or more cell culture(s) (2) located on the bottom of the receptacle can be positioned, and in some cases in that position can be brought into such close proximity to said cell culture, as to respectively laterally displace a partial volume of the liquid culture medium (4) which covers the cell culture.
4. A device in accordance with one of the Claims 1 to 3, characterized in that one sensor, or a multiplicity of sensors (6), is/are placed on, or in, the bottom of the receptacle (3a) and in that culture areas separated from one another, are formed especially by means of the application of structured, cell repellent coatings or created by

means of a three dimensional contouring of the said bottom with recesses or elevations about the separated areas therebetween, and in that the culture medium (4) in the culture areas advantageously is respectively present as drops (25).

5. A device in accordance with one of the Claims 1 to 4, characterized in that the bottom of the receptacle is formed by at least one part of at least one of the wafers which possess the sensor(s) 6.

6. A device in accordance with the generic concept of Claim 1, characterized in that an open top container, is provided as a receptacle (3), into which a separating element (7) extends, which divides the space of the entire receptacle (3) into two space portions, one positioned above the other, and in that the bottom side space portion forms a small volume reaction space (8) in comparison to the entire volume of the receptacle (3) and the other space portion forms a reservoir (14) and in that at least one flow channel (9) is provided, which first, communicates with the reaction space (8) and second, communicates with the reservoir space (14) and in that within the separating element (7) one or more through-flow channels (15) are provided, which open into the small volume reaction space (8) of the receptacle (3) and/or in the reservoir space (14) of the receptacle (3).

7. A device in accordance with one of the Claims 1 to 6, characterized in that one or more sensors (6) and/or measurement apparatuses are placed in the area of the reaction space (8, 8a).

8. A device in accordance with Claim 6 or 7, characterized in that the separating element (7) within the receptacle (3) is movable, back and forth, between a position proximal to the bottom and a position remote from the bottom and in the

position proximal to the bottom borders the reaction space 8.

9. A device in accordance with one of the Claims 1 to 8, characterized in that the side of the separating element (7) proximal to the bottom, possesses a cover, that is, a bordering surface, for the measurement of the substances consumed or produced by the cells, especially a surface corresponding to the sensor surface.

10. A device in accordance with one of the Claims 6 to 9, characterized in that the separating element (7) can be inserted from above into the receptacle (3).

11. A device in accordance with one of the Claims 1 to 10, characterized in that the distance of the separating element (7) from the cell culture (2) and therewith the bottom proximal position of the said separating element (7) is adjustable.

12. A device in accordance with one of the Claims 6 to 11, characterized in that the separating element (7) is advantageously constructed in the shape of a hand stamp and has a head (10) with approximately the same cross-section as that of the receptacle (3), and which separating element (7) divides the said receptacle into a reaction space (8) and a reservoir (14) and in that on the separating element (7) a shaft (11) is connected which extends to the outside, the outside cross-section of which shaft (11) is smaller than the open inside cross-section of the receptacle 3 and in that the intervening space between the shaft and the inner wall of the receptacle (3) forms the reservoir (14).

13. A device in accordance with one of the Claims 6 to 12, characterized in that within the separating element (7) one or more flow channels are provided which first, open in the reaction space (8) and second open in the reservoir space (14).

14. A device in accordance with one of the Claims 6 to 13, characterized in that the flow channel (9) is formed by an annular gap provided between the separating element (7) and the inner wall of the receptacle (3) or is designed as a rim profiling, and in that this flow channel, or flow channels remain(s) available, in the case of a separating element (7) which can be adjusted as to height, at least within the lift interval between a position proximal to the bottom and a position remote therefrom.

15. A device in accordance with one of the Claims 1 to 14, characterized in that the underside of the separating element (7) possesses a contouring for the guidance of gas bubbles to the outside, this being preferably a convex bulging.

16. A device in accordance with one of the Claims 6 to 15, characterized in that a thrust limitation for the separating element (7) is provided and in that for this purpose, in the bottom proximal position an effective restraint is placed on the separating element (7), preferably this being a detent abutted by the upper rim (12) of the receptacle (3).

17. A device in accordance with Claim 16, characterized in that the detent placed on the upper receptacle rim (12) by the separating element (7) is made by means of a cover (13) which overlaps the rim (12) of the receptacle (3) or alternately by a cover (13) with a conical section which engages in a counter conical section of the receptacle opening.

18. A device in accordance with one of the Claims 1 to 17, characterized in that the separating element (7) possesses on its upper side, an especially standard opening, preferably of a receiving conical shape for the coupling with a pipette, a pipette tip, or a dispenser channel.

19. A device in accordance with one of the Claims 6 to 18, characterized in that the receiving volume of the reservoir (14, 14a) is multiply greater than the receiving volume of the reaction space (8, 8a) and that these two volumes are in relation to one another by the ratios of respectively, 10 : 1 to approximately 100 : 1.

20. A device in accordance with one of the Claims 1 to 19, characterized in that at least on the bottom of the receptacle (3) or a receiver, is placed at least one chip with one or preferably several microsensors.

21. A device in accordance with one of the Claims 1 to 20, characterized in that on the separating element (7), proximal to the reaction space (8) and/or to the reservoir (14), sensors (6) and/or electrodes are provided.

22. A device in accordance with one of the Claims 6 to 21, characterized in that at a distance from the receptacle bottom and between this and the separating element (7) in its bottom proximal position, a microporous membrane (23) or a similar filter or protective covering for the cell culture is provided.

23. A device in accordance with one of the Claims 1 to 22, characterized in that the separating element (7) is comprised of a smooth, cell rejecting, inert and easily sterilized material, this being preferably polytetrafluor ethylene.

24. A device in accordance with one of the Claims 1 to 23, characterized in that the resting surface for the cell culture (2) is optically transparent and in that the resting surface is related to an optical measuring apparatus, which, preferably, is situated on the underside.

25. A device in accordance with one of the Claims 1 to 24, characterized in that a plurality of receptacles (3) are provided, preferably as a part of a pipetting automat (19) and in that these receptacles (3) are especially made by commercially available multiwell plates (20) and in that on the lower ends of the dispenser channels of the pipetting automat, respectively, the separating element (7) is provided.

26. A device in accordance with one of the Claims 1 to 25, characterized in that the separating element (7) on its end distal to the bottom, possesses a fitting for connecting to, or plugging into, a dispenser channel (22), preferably a pipetting automat.